

Pesticide Assessment Guidelines
Subdivision F, Position Document
Selection of a Maximum Tolerated
Dose (MTD) in Oncogenicity Studies

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SELECTION OF A MAXIMUM TOLERATED DOSE (MTD) IN ONCOGENICITY STUDIES

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The Toxicology Branch of the Office of Pesticide Programs frequently encounters oncogenicity and chronic feeding studies in which the dose levels selected for toxicological evaluation were not sufficiently high to evoke significant signs of toxicity in the test animals. The failure to observe toxicity in an oncogenicity study at the highest dose level may compromise the hazard identification of the oncogenic activity of the compound because the test animal may not have been sufficiently challenged under the conditions of the study.

Despite general agreement among scientists regarding the requirements for a MTD, there appears to be a lack of consensus regarding the toxic end points which are adequate for fulfilling a MTD in an oncogenicity study. In fact, the term MTD has been described in as many different subjective terms as there are individuals who use it. This has frequently led to confusion and differences in the interpretation of results between regulatory agencies and investigators at the termination of these studies.

This problem has not gone unnoticed; several efforts, primarily via the National Toxicology Program and OSTP are being expended to describe more clearly the MTD concept and to bring consistency to the definition.

Within the various guidelines for oncogenicity studies which have been developed by the Environmental Protection Agency, there are apparent differences in the description of the MTD concept. The 1978 proposed guidelines issued by the Office of Pesticide Programs provided a minimal definition of the MTD, whereas the 1982 final guidelines do not employ the term. In response to the need to improve scientific and regulatory decisions and to provide additional guidance for the investigators performing oncogenicity studies, this position statement is presented by the Toxicology Branch of the Hazard Evaluation Division in the Office of Pesticide Programs.

The Office of Pesticide Programs position statement should be considered to be an addendum to the Program's 1984 toxicity testing guidelines. This position statement reflects the scientific thoughts of only the Office of Pesticide Programs and should not be construed to be guidance from any other Office within EPA. It should not be assumed that a study found acceptable by the Office of Pesticide Programs in terms of the highest dose tested will be automatically acceptable to other offices in EPA. It is anticipated that a global Agency definition of MTD will be developed in the future by the Risk Assessment Forum in EPA.

The Office of Pesticide Programs believes that in evaluating the acceptability of chronic/oncogenicity studies, consideration should be given to the concept that the MTD is a predicted value derived from observed toxicities in subchronic studies.

The highest dose to be tested in the oncogenicity study should be selected below a level which resulted in significant life-threatening toxicity in the subchronic study. The level should not be selected too far below a life threatening level because the highest dose tested in the oncogenicity study should elicit significant toxicity without substantially altering the normal life-span of the test species from effects other than tumor formation. This dose selection process may be difficult and we advise sponsors to seek some guidance from the Program before proceedings with the chronic study.

Changes in several toxicological parameters observed in subchronic studies or chronic studies are important in determining the highest dose to be tested in the oncogenicity study or in determining that the highest level of testing in the chronic study was adequate. These changes involve body weight gain decrement, hematological effects, histopathologic changes, alterations in clinical chemistries and urinalysis, organ weight changes, neurological symptoms and other clinical

signs. Attempts should be made when possible to correlate biochemical parameters with clinical signs of toxicity or histopathological changes in tissues or organs showing a toxic response to the chemical under test. The Office of Pesticide Programs would like to present some examples of some types of toxicity observed in subchronic or chronic studies which may used to select the highest dose level to be tested or that the highest dose tested sufficiently expressed the full range of toxicity of the test substance. These examples are not intended to be all inclusive by any means.

Body Weight Effects:

The highest dose to be tested can be established on the basis of a biologically significant decrement in body weight gain. The Office of Pesticide Programs believes this decrement in body weight gain should reach 10-15% in subchronic testing.

When a clinical sign such as weight loss is observed, attempts should be made to determine if this is caused by anorexia, palatability, dysphagia or secondary effects due to diarrhea, diuresis or nutritional imbalance. Edema or organ weight gain, particularly with the liver, may mask a body weight gain decrement.

Histopathology:

Consideration of pathological changes in predicting a MTD and establishing the highest dose to be tested in the oncogenicity study is imperative especially if these toxicities may be expected, upon chronic exposure, to result in shortening the lifespan of the animal, thereby decreasing the time on test.

In the subchronic study dose levels of the agent should be used which demonstrates a spectrum of histopathological changes in tissues such as fatty metamorphosis, vacuolization, mineralization, cell proliferation, cell degeneration, cell necrosis and cell death.

One word of caution should be made at this point. In several instances during the last two years the Program has seen the highest dose to be tested in the chronic study selected on the basis of organ weight increases, cloudy swelling, fatty metamorphosis and vacuolation, alone or in combination. In many of these instances the Program believes that the highest dose selected was inadequate. Fatty metamorphosis of the liver is often not life-threatening to the experimental animals and the high dose selected in these studies was set at the lower end of dosages shown to produce fatty changes. The Program believes that the highest dose to be tested for a hepatotoxin should be set at a upper level of

dosages associated with fatty metamorphosis. If necrosis is seen along with fatty metamorphosis then a level should be selected that will probably produce some minimal degree of necrosis which would not effect the survivability of the animals on test.

Hematologic effects:

Biologically significant alterations indicating anemia, changes in prothrombin clotting time, leucopenia, leukemia or significant alteration in the relative number of critical formed elements can be used to make a prediction regarding the highest dose to be tested in the oncogenicity study and the likelihood of the survivability of the high dose animals to the termination of the study or to establish that the high dose employed in the chronic study was adequate.

Clinical chemistries:

Significant depression of at least two of the assayed cholinesterase enzyme measurements, i.e., plasma, red blood cell or brain acetylcholinesterase levels can be used to establish the highest dose to be tested or that the high level employed in the chronic study was adequate.

Significant alterations in clinical chemistries such as aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase or blood urea nitrogen can be used to support the selection of the highest dose to be tested when they correlate with histopathologic changes in seen tissues or organs.

Urinalysis:

Changes in urinalysis can add support to the selection of the proper high dose level to use in the chronic study or can support the adequacy of the high dose used in the completed oncogenicity study.

Organ weight changes:

Biologically significant alterations in organ weights will support the selection of the high dose to be tested or the adequacy of the doses used in an oncogenicity if they correlate with significant histopathology seen in that organ. The MTD will not be considered to be reached if increases in relative liver to body weight ratios occur in the absence of significant histopathology in the liver; these increases are more likely to result from microsomal enzyme induction, representing an adaptive or pharmacologic response of the liver to the test chemical and not likely to effect survivability.

Basically, the Office of Pesticide Programs will consider a oncogenicity study to be adequately performed if the highest dose level studied vigorously tests the agent for oncogenic potential at levels somewhat below test levels which might compromise survivability.

After the highest dose to be tested has been selected, at least two doses lower than the predicted MTD should be selected not only to help validate responses seen at the highest dose level as well as to develop dose-response curve information but also to provide a margin of safety against overestimating the MTD. Thus, in a three dose study, if survivability is poor at the highest dose tested, the mid-dose level, set approximately at one-half the MTD, could substitute as an adequate level to test the potential oncogenicity of the test agent.

A MTD level should be determined for each species, sex and strain of animal to be used in an oncogenicity study. If the doses selected for each sex are reasonably close, then the doses should be the same.

In oncogenicity studies of pesticides, a dose of 1 gram/kg of body weight/day should provide an adequate upper limit for the testing of most pesticides in laboratory animals. This

upper limit of 1 gram/kg applies to chemicals which do not produce toxicological effects in subchronic studies. The dose of 1 gram/kg/day is equivalent to dietary concentrations of approximately 20,000 ppm (i.e., 2%) in the rat and 7,000 ppm (i.e., 0.7%) in the mouse. The dose level of 1 gram/kg/day is considered an adequate upper limit for testing pesticides because of exposure is generally limited by their relative potency and selective toxicity.

There are approximately 250 pesticides that have been registered for some use on food commodities. In no case does the exposure of the general U.S. population exceed one milligram per kilogram of body based on cumulative total maximum residue concentration values. The cumulative total maximum residue concentration represents the theoretical residues levels from all commodities treated with a pesticide at the time it leaves the farm and assumes the maximum use of the agent in terms of label recommendations. Reduction in the level of a pesticide because of decomposition, processing of the commodity, food preparation and cooking is not considered in these values. Therefore, these values are often much higher than the real world human exposure to pesticides. The vast majority of agents are considerably below a human exposure level of one milligram per kilogram in terms of their cumulative total maximum residue concentration.

The Program believes that the 1000 milligram per kilogram level established for the rat and mice is a realistic higher bound for testing of a pesticide for oncogenic potential. Additionally, in the past three years the Office of Pesticide Programs' Peer Review Committee has evaluated more than 50 agents for carcinogenic potential. In only two cases were possible carcinogenic effects seen only at levels exceeding 1000 milligrams per kilogram. In one situation urinary bladder tumors were seen at 40,000 ppm probably caused by urinary caculi. In the other case adenomas/carcinomas were seen in the male rat kidney at 30,000 ppm and was considered to be an equivocal finding by the Programs' Scientific Advisory Panel.

If the anticipated toxicity at the properly selected high dose was not seen because adaptation occurred in the chronic study, the Office of Pesticide Programs would consider that chronic study as having met our scientific standards and it would not have to be repeated because of an absence of significant toxicity at the termination of the oncogenicity study. To the contrary, a chronic/oncogenicity study without a demonstrated effect would be considered unacceptable as an oncogenicity test if the highest dose was not predicted from observed toxic effects in a subchronic study.

It is strongly advised that the sponsor present, in writing, the scientific rationale for the highest dose to be tested in an oncogenicity study and whenever possible to meet with toxicologists in the Office of Pesticide Programs to reach some concurrence on the doses to be tested in the study before the initiation of the study. This interaction between the Agency and investigators could avoid future problems in evaluating oncogenicity studies in which inappropriate dose levels were used for the testing of the carcinogenic potential of a compound.